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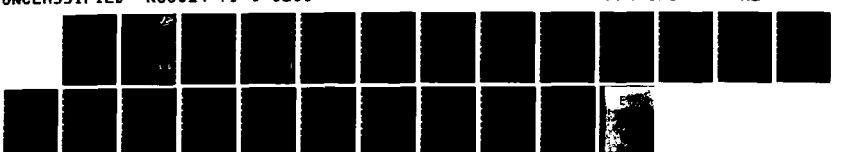

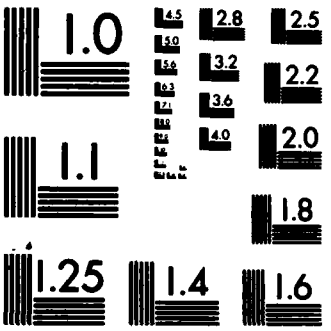

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THE EFFECT OF AN ELECTRICAL LEFT VENTRICULAR ASSIST DEVICE ON RED BLOOD CELL AND PLATELET SURVIVAL IN THE COW

by

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
Blood volume measurements were made in cows after infusion of human ^{125}I albumin and autologous ^{51}Cr-labeled red blood cells. Repeated intravenous infusions of iodinated human albumin did not appear to isosensitize the cows. When the cow red blood cells were incubated at 37 C after labeling with ^{51}Cr, there was elution of the ^{51}Cr, and the ^{51}Cr T_{50} values were 45 hours in both healthy cows and cows with LVAD's.

Measurements also were made in the cow platelets labeled with ^{51}Cr or ^{111}Indium-

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oxine. The platelets labeled with ^{51}Cr had T_{50} values of 4 days, and platelets labeled with ^{111}Indium-oxine had T_{50} values of 0.9 to 2.7 days. ^{51}Cr-labeled platelets had similar T_{50} values in healthy cows and cows with LVAD's.

Bovine platelets isolated from units of blood using serial differential centrifugation were labeled with ^{51}Cr or with ^{111}Indium-oxine, and after infusion in healthy cows and cows with LVAD's measurements were made of platelet circulation and distribution. The disappearance of platelet radioactivity from the blood was linear with time, and the platelet lifespan was 6-10 days. The presence of an LVAD did not affect initial recovery or lifespan of cow platelets.

ABSTRACT

Blood volume measurements were made in cows after infusion of human ^{125}I albumin and autologous ^{51}Cr-labeled red blood cells. Repeated intravenous infusions of iodinated human albumin did not appear to isosensitize the cows. When the cow red blood cells were incubated at 37 C after labeling with ^{51}Cr, there was elution of the ^{51}Cr, and the ^{51}Cr T_{50} values were 45 hours in both healthy cows and cows with LVAD's.

Measurements also were made in the cow platelets labeled with ^{51}Cr or ^{111}Indium-oxine. The platelets labeled with ^{51}Cr had T_{50} values of 4 days, and platelets labeled with ^{111}Indium-oxine had T_{50} values of 0.9 to 2.7 days. ^{51}Cr-labeled platelets had similar T_{50} values in healthy cows and cows with LVAD's.

Bovine platelets isolated from units of blood using serial differential centrifugation were labeled with ^{51}Cr or with ^{111}Indium-oxine, and after infusion in healthy cows and cows with LVAD's measurements were made of platelet circulation and distribution. The disappearance of platelet radioactivity from the blood was linear with time, and the platelet lifespan was 6-10 days. The presence of an LVAD did not affect initial recovery or lifespan of cow platelets.

INTRODUCTION

There are a number of double-valved left ventricular assist devices (LVADs) being used in patients with reversible ventricular dysfunction or with myocardial fibrosis who require prolonged circulatory support.[1,2] Preliminary studies have shown that patients with pneumatic LVADs who were on cardiopulmonary bypass for 3-6 hours prior to LVAD implantation developed a diffuse bleeding diathesis and profound thrombocytopenia.

Biosynthesis materials cover the inner surface of the pump housings and the blood-contacting diaphragms, and the interaction of these materials with the blood products that pass over them is not well understood.

Red cell and platelet survival measurements were made in cows before and after the implantation of an electrical LVAD to determine whether the interaction of these blood products with the pump and/or the biosynthetic surface would result in damage that would shorten their lifespan.

MATERIALS AND METHODS

The Left Ventricular Assist Device

An electrically-energized blood pump was implanted in cows weighing 80 to 90 kg. Pulsatile blood flows were maintained continuously (4.0-5.0 L/minute) until such time as the animal was sacrificed (30 to 149 days postoperatively). The LVAD consisted of a pusher-plate blood pump and a 50 volt low-speed torque motor encased in a titanium housing (10.5 cm diameter, 4.0 cm width) weighing 800 grams. Flocked polyester fibrils, 25 um in diameter and 250-300 um in length, covered the inner surface of the pump housing and the blood-contacting diaphragm. In some experiments, the blood-contacting surface was seeded with culture fibroblasts which had been grown from unrelated donor connective tissue specimens.

Each LVAD had an inflow and an outflow conduit consisting of a woven Dacron graft (25 mm in diameter) and inflow and outflow valves from Porcine xenograft aortic valves. All animals were maintained on enough sodium warfarin to prolong their prothrombin times to two times the normal level throughout the study.

A 450 ml unit of bovine blood anticoagulated with 15% ACD was centrifuged at 22 C at 4500 X g for 2.5 minutes to prepare platelet-rich plasma and a red cell concentrate. The red cell concentrate was diluted with an equal volume of 0.9% NaCl, incubated with 100 uCi of sodium chromate (0.5 uCi ^{51}Cr per ml of red cells) at 37 C for 30 minutes, and then stored in a 600 ml polyvinylchloride (PVC) transfer pack in a 37 C

water bath for 64 hours.

Plasma volume was measured with ^{125}I human albumin as previously described.[3] Red blood cell volume was measured using fresh autologous ^{51}Cr-labeled red blood cells.[4] Blood samples were obtained prior to and 5, 10, 15, 20, 25, 30, and 60 minutes after infusion of the ^{125}I-labeled albumin and ^{51}Cr-labeled red blood cells. Total blood volume was the sum of the ^{125}I plasma volume and the ^{51}Cr red cell volume. Elution of the ^{51}Cr label from red blood cells and platelets was studied. Erythrocyte survival studies were performed prior to and following LVAD implantation by transfusing the cows with fresh autologous ^{51}Cr-labeled red blood cells and following the disappearance of radioactivity. The red cell T_{50} was calculated by exponential linear regression. Samples were obtained periodically for measurement of total ^{51}Cr radioactivity per volume of diluted red cells, supernatant ^{51}Cr radioactivity, supernatant K^+, supernatant hemoglobin, and hematocrit.

Percent red blood cell ^{51}Cr uptake and percent red cell ^{51}Cr after various intervals of storage were calculated using the following formula:

$$\% \text{ Red blood cell } {}^{51}Cr = \frac{{}^{51}Cr \text{ in RBC Suspension} - \text{Supernatant } {}^{51}Cr}{{}^{51}Cr \text{ in RBC Suspension}} \times 100$$

$$= \frac{{}^{51}Cr \text{ in Suspension} - (1 - Hct)(\text{Supernatant } {}^{51}Cr/\text{ml supernatant})}{{}^{51}Cr \text{ in RBC Suspension}} \times 100$$

Platelet survival studies were performed prior to and following LVAD implantation. In some studies the platelets were labeled with ^{51}Cr and in other studies the platelets were labeled with ^{111}Indium-oxine. The methods for labeling and determining the amount of platelet-associated radioactivity injected and the survival of radiolabeled platelets have

been described previously.[5,6] The platelet T_{50} was determined by linear regression.

Platelet-rich plasma was isolated from 450 ml of whole blood collected in acid-citrate-dextrose (ACD, NIH, Formula A). A volume of ACD equivalent to 8% of the volume of platelet-rich plasma was added. The platelet-rich plasma-ACD mixture was centrifuged at 4500 X g for 5 minutes. All but 30 ml of the platelet-poor plasma was expressed from the bag and the platelet concentrate was stored undisturbed at room temperature for 30 minutes.

The platelet concentrate was incubated with 300 uCi of ^{51}Cr (sodium chromate) for 30 minutes at 22 $\pm$ 2 C, diluted with 100 ml of autologous plasma and 10% ACD, and centrifuged at 4500 X g for 5 minutes. The supernatant fluid was removed, and the platelets were resuspended in 60 ml of autologous plasma.

The labeled resuspended platelet concentrate was stored in a 300 ml PVC transfer pack in a 37 C water bath for 64 hours. Samples were obtained for determination of total ^{51}Cr radioactivity, supernatant ^{51}Cr radioactivity, and for platelet counts. Percent platelet uptake and percent platelet ^{51}Cr after various intervals of storage were calculated as follows:

$$\text{Percent Platelet } ^{51}\text{Cr Uptake} = \frac{^{51}\text{Cr in Labeled Suspension}}{^{51}\text{Cr in Labeled Suspension} + ^{51}\text{Cr in Waste}} \times \% \text{ Platelet } ^{51}\text{Cr in Labeled Suspension}$$

$$\text{Percent Platelet } ^{51}\text{Cr After Storage} = \frac{\text{Total } ^{51}\text{Cr in Platelet Suspension} - \text{Supernatant } ^{51}\text{Cr}}{\text{Total } ^{51}\text{Cr in Platelet Suspension}}$$

RESULTS

Blood Volume Determination Using Human ^{125}I Albumin and ^{51}Cr Labeled Autologous Red Blood Cells

Plasma volume was measured with human ^{125}I albumin on 7 occasions in each of 5 animals (Table 1). When determined from the plasma sample obtained 15 minutes after ^{125}I albumin injection, plasma volume averaged 5720 ml (56.8 ml/kg). The plasma volume increased by only 6.5% when the 30-minute determination was compared with the 5-minute determination, indicating that distribution of ^{125}I albumin into the extravascular space during that period was minimal. Plasma volume determined from the 60-minute post-infusion sample was 36% greater than that determined from the 5-minute sample.

Red blood cell volume was measured with ^{51}Cr-labeled autologous red cells on 5 occasions in 3 animals with LVAD's in place and 2 without (Table 1). Red blood cell volume averaged 1660 ml (15.9 ml/kg) when determined from the 15-minute post-infusion sample, and increased progressively during the 15- to 60-minute sampling interval (14.4% increase at 30 minutes, and 33% increase at 60 minutes).

The f factor was used to estimate the total body hematocrit from the peripheral venous hematocrit; the total body hematocrit was derived from the measured ^{51}Cr red cell volume divided by the total of the measured ^{51}Cr red cell volume and the ^{125}I albumin measured plasma volume. The "f" factor was calculated from the 10-, 15-, and 20-minute values of peripheral venous hematocrit and the total body hematocrit estimated from the ^{125}I

albumin plasma volume and ^{51}Cr labeled red cell volume values. The f factor was similar at each of the three sampling times, and averaged 0.808 (n = 13 measurements in 5 animals (Table 1)).

The blood volume in each of 4 cows was calculated simultaneously from the f factor and the ^{51}Cr red blood cell volume and the f factor and the ^{125}I albumin plasma volume (Table 2), with similar results. The total blood volume increased by about 20% during the 60 minutes after injection of the ^{51}Cr-labeled red blood cells and ^{125}I albumin.

Plasma Volume Measurement Using Human ^{125}I Albumin

On 3 separate occasions, cow 577 green was given an intravenous injection of 2.5 ml of human albumin labeled with 0.4 uCi of ^{125}I. An interval of 28 days passed between the first and second injections, and 98 days between the second and third injections. A plasma volume determination was made on each occasion by measuring the dilution of the ^{125}I albumin in the plasma volume 15 minutes after injection.

Elution of ^{51}Cr Label from Red Blood Cells

The initial uptake of ^{51}Cr during 37 C storage was 95% (Table 3). Thereafter, there was a progressive loss of ^{51}Cr, until after 64 hours of storage at 37 C the percent of total ^{51}Cr localized on the red blood cells had decreased to 72%, representing a 23% loss of red blood cell ^{51}Cr radioactivity. During the period of red blood cell storage, there were only minimal increases in supernatant hemoglobin and K^+, and the hematocrit remained stable, demonstrating that the loss of ^{51}Cr did not result from red blood cell hemolysis. These data indicate a significant degree of elution of ^{51}Cr from labeled bovine red blood cells stored at

37 C.

Stability of ^{51}Cr Relative to Platelet Count in Labeled Platelets Incubated In Vitro at 37 C

Table 4 shows the changes in platelet ^{51}Cr radioactivity and platelet count during storage of ^{51}Cr-labeled platelet concentrates at 37 C for 64 hours. In the labeled-washed-resuspended platelet concentrate, initially about 70% of the ^{51}Cr radioactivity was associated with cells and 30% was free in the supernatant plasma. After 64 hours of storage only 41% of the radioactivity was associated with cells, a decrease of 41%. Platelet counts measured during the initial 23.5 hours of storage showed a decrease of 41%, during which time the ^{51}Cr cell radioactivity decreased by 15%. These findings raise the possibility that the loss of platelet radioactivity during in vitro storage at 37 C may have been due to platelet lysis rather than to elution of the label.

Red Cell Survival Studies

Red cell survival studies were performed in one cow prior to LVAD implantation and in 5 cows 13 to 34 days following implantation (Table 5). There was no difference in T_{50} values (45 hours) between animals with or without LVADs, and the rapid elution of ^{51}Cr from the red blood cells may or may not have been a factor in the very short T_{50} values.

Platelet Survival Studies

Measurements of ^{51}Cr platelet survivals were made in 2 cows prior to implantation, and in 3 cows 7 to 136 days following implantation (Table 6). ^{111}In platelet survival measurements were made in 3 cows 20 to 60 days following LVAD implantation (Table 7).

Processing of the radiolabeled platelets to determine the platelet-associated radioactivity injected resulted in a loss of ^{51}Cr and ^{111}In activity from the platelets. The platelet-associated radioactivity recovered immediately following transfusion at zero time was assumed to be 100%. There were no significant differences in the platelet T_{50} values between animals with and without LVADs when the platelets were labeled with ^{51}Cr. Cow platelets labeled with ^{111}In demonstrated a wide range in T_{50} values that were shorter than those observed with ^{51}Cr suggesting an elution of ^{111}In from the platelets.

DISCUSSION

We have demonstrated that accurate measurements of plasma volume can be made in the cow using ^{125}I human albumin, with no evidence of immunization to the human albumin. A similar observation has been made in the baboon.[7] The cow's total blood volume was estimated from the ^{125}I plasma volume and the total body hematocrit (venous hematocrit X an f factor (0.808) which was derived from the total body hematocrit estimated by simultaneous measurement of the ^{125}I plasma volume and the ^{51}Cr red cell volume).

^{51}Cr elutes from the cow red cell in vitro and in vivo, and this elution produces a marked shortening of the red cell T_{50} value. The T_{50} values in 5 cows with LVADs and in one without suggested reproducible in vivo elution patterns.

When ^{51}Cr was used to label the platelets, T_{50} values were reproducible, whereas the T_{50} values obtained with ^{111}In-oxine were variable, suggesting that ^{111}In eluted from the cow platelets during circulation. Unlike our finding in baboon studies,[6] ^{51}Cr proved to be more satisfactory for labeling cow platelets than ^{111}In-oxine.

Our data suggest that the blood surface of the LVAD did not cause enough damage to shorten the red cell or platelet lifespan.

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TABLE 1

MEASUREMENT OF PLASMA VOLUME USING HUMAN ^{125}I-ALBUMIN AND RED BLOOD CELL VOLUME USING AUTOLOGOUS ^{51}Cr LABELED RBC's

COW	PLASMA VOLUME(ml) 5 min	10 min	15 min	20 min	25 min	30 min	60 min	RED BLOOD CELL VOLUME (ml) 5 min	10 min	15 min	20 min	25 min	30 min	60 min	"f" FACTOR 10 min	15 min	20 min
#577 10/3/79 (Normal animal)	ND	4140	4550	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	____	____	____
#577 10/31/79 (Normal animal)	3890	4240	4450	ND	ND	4980	ND	1050	1070	1090	1080	1170	1130	ND	0.789	0.781	0.778 (30')
#577 2/6/80 (Normal animal)	ND	ND	5020	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	____	____	____
#583 2/7/80 (Normal animal)	5290	5290	ND	5710	ND	5900	ND	2040	2090	ND	2160	ND	2240	2370	.953	____	.920
#622 10/29/80 (LVAD 10/-/80)	8230	8090	7850	9150	8290	7930	9190	1710	2210	2290	2340	2300	2250	2610	.814	.856	.768
#620 10/29/80 (LVAD 9/25/80)	6600	5260	6360	6170	6640	6500	7570	1590	1860	1860	1880	1970	2070	2060	.800	.788	.810
#625 12/8/80 (LVAD 11/7/80)	ND	6070	6084	6120	ND	6650	7820	ND	1350	1400	1480	ND	1450	1490	.720	.737	.764
MEAN	6000	5680	5720	6790	7460	6390	8190	1600	1720	1660	1790	1810	1830	2130	.815	.790	.808
SD																	
N																	

ND=Not Measured

TABLE 2

BLOOD VOLUME DETERMINED SIMULTANEOUSLY FROM THE ^{51}Cr RED BLOOD CELL VOLUME AND THE TOTAL BODY HEMATOCRIT AND FROM THE ^{125}I PLASMA VOLUME AND THE TOTAL BODY HEMATOCRIT

	5	10	15	20	25	30	60
TOTAL BLOOD VOLUME ESTIMATED FROM ^{51}Cr VOLUME AND THE TOTAL BODY HEMATOCRIT	7420 ± 418	8250 ± 1230	8700 ± 1500	8630 ± 1330	9450 ± 1200	8720 ± 1000	9590 ± 1780
TOTAL BLOOD VOLUME ESTIMATED FROM ^{125}I ALBUMIN PLASMA VOLUME AND THE TOTAL BODY HEMATOCRIT	8790 ± 1600	8320 ± 1430	8600 ± 1360	8810 ± 2000	9660 ± 1480	8750 ± 980	10430 ± 1280

Mean ± 1 S.D.

TABLE 3

ELUTION OF ^{51}Cr FROM BOVINE RED BLOOD CELLS STORED AT 37 C FOR 64 HOURS

TIME	^{51}Cr IN RBC SUSPENSION (CPM/2 ml)	^{51}Cr IN SUPERNATANT (CPM/2 ml)	% OF TOTAL RADIOACTIVITY ASSOCIATED WITH RBC	HCT (V%)	SUPERNATANT HEMOGLOBIN (mg %)	SUPERNATANT K+ (meg/L)
PRE-LABEL					17	1.4
0	107200	7730	95.0%	30%	15	1.7
70 MIN	101540	9058	93.8%	30%	14	2.0
130 MIN	101700	10060	93.1%	30%	21	2.3
16 HOURS	101270	18490	87.2%	30%	21	3.6
23.5 HOURS	99810	20210	79.8%	30%	23	4.0
40 HOURS	99040	23380	76.4%	30%	34	4.5
50 HOURS	95530	24110	74.8%	ND	89	4.6
64 HOURS	91980	25870	71.9%	ND	110	4.8

ND = NOT MEASURED

TABLE 4

PLATELET ^{51}Cr RADIOACTIVITY AND PLATELET COUNTS IN ^{51}Cr LABELED PLATELET CONCENTRATE STORED AT 37 C FOR 64 HOURS

TIME	^{51}Cr IN PLATELET SUSPENSION (CPM/ml)	^{51}Cr IN SUPERNATANT OF PLATELET CONCENTRATE (CPM/0.5 ml)	% TOTAL ^{51}Cr ON PLATELET	PLATELET COUNT per mm^3
0	57690	8716	69.8%	4.12×10^6
70 MIN	58610	9141	68.8%	5.31×10^6
130 MIN	58710	9865	66.4%	4.07×10^6
16 HOURS	58450	11270	61.4%	2.70×10^6
23.5 HOURS	57390	11640	59.4%	2.61×10^6
40 HOURS	56940	15580	45.7%	ND
64 HOURS	55380	16250	41.3%	ND

ND = NOT MEASURED

TABLE 5

^{51}CR RBC SURVIVALS

CONTROLS		15 MIN	20 MIN	30 MIN	1 HR	24 HRS	48 HRS	7 DAYS	14 DAYS	T_{50}
COW 583		101%	98%	94%	89%	60.5%	—	36.5%	—	45.9 HRS
COW 360		97.5%	99.5%	94%	87.5%	58%	48%	30%	—	43.2 HRS
MEAN										44.6 HRS
LVAD COWS	DAYS FOLLOWING IMPLANTATION									
COW 620	34	100%	99.5%	92.5%	90.0%	60.0%	48.0%	32.5%	18%	42.1 HRS
COW 622	29	102%	98.5%	104.5%	91.0%	56.0%	49.0%	26.0%	—	45.3 HRS
COW 625	19	99.5%	94.5%	96.0%	95.0%	65.5%	50.0%	24.5%	9%	44.4 HRS
COW 630	13	100%	92.5%	87.5%	80.5%	53.5%	43.5%	29.0%	16%	39.1 HRS
COW 631	13	100%	97.5%	98.0%	93.0%	62.0%	48.0%	29.0%	23%	41.9 HRS
MEAN										42.4
SD										2.5

TABLE 6

^{51}CR PLATELET SURVIVALS

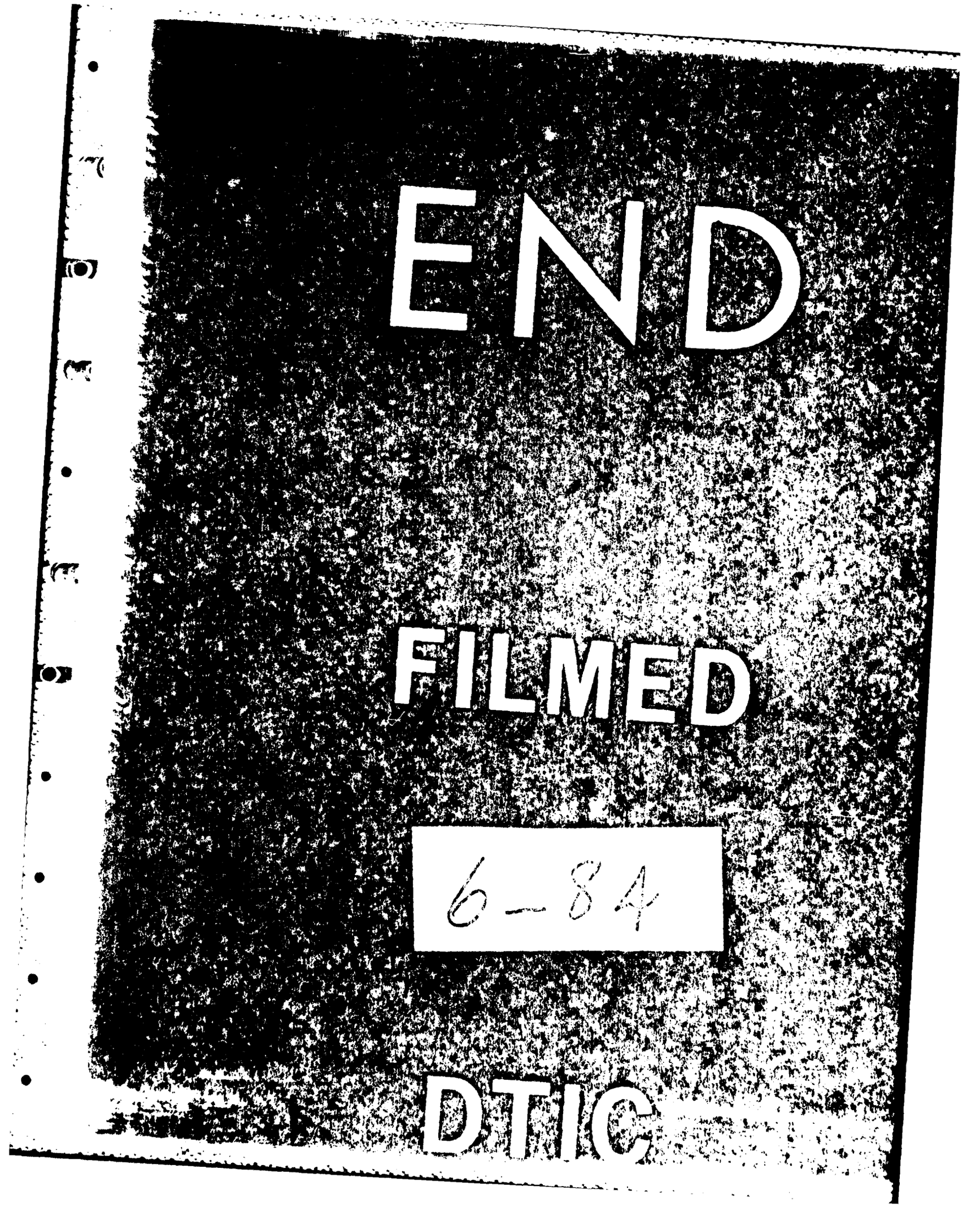
CONTROLS	0 HR	24 HR	48 HR	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY	T_{50}
COW 577 #1	100	74%	54%			16%			2.9 DAYS
COW 577 #2	100	96%	86%			46%	43%	35%	5.10 DAYS
COW 359	100	94%	52%	40%			9%		3.05 DAYS
MEAN									3.68
SD									±1.00

LVAD COWS	DAYS FOLLOWING IMPLANTATION								
COW 631	100	63%	63%			17%	19%	4%	3.54 DAYS
COW 649	100	39%				8%	2%	3%	3.17 DAYS
COW 652	100	84%	65%	28%				14%	3.65 DAYS
MEAN									3.45
SD									±0.25

TABLE 7

^{111}IN PLATELET SURVIVALS

LVAD COWS	DAYS FOLLOWING IMPLANTATION	0 HR	24 HR	48 HR	3 DAY	4 DAY	5 DAY	6 DAY	7 DAY	T_{50}
COW 620		100	43.0							0.88 DAYS
COW 630		100	73		12		5	3		2.68 DAYS
COW 631		100	71	41						1.7 DAYS
MEAN										1.75
SD										±0.9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